REMARKS

Status of the Claims

Claims 1-24 and 26-28 and 30-45 are currently pending in the application. Claims 21-30, 32 and 39 stand rejected. The Examiner objects to claims 31 and 33-38. Claims 1-20, 40 and 41 are withdrawn as being drawn to a non-elected invention. Claims 1-24, 26, 28, 30, 31, 32, 34, 36, 37 and 38 have been amended. Claims 25 and 29 have been cancelled. All amendments and cancellations are made without prejudice or disclaimer. New claims 42-45 have been added. No new matter has been added by way of the present amendments. Specifically, the amendments to the claims are to conform the claims more closely to US practice and add the limitation "*Bombyx mori*" which is supported throughout the specification. Furthermore, amendments to claims 21-23 are supported at least at page 17, lines 6-12, page 21, lines 2-4 of the specification and the drawings. The amendment of claim 38 is supported throughout the specification at, for instance, paragraph [0081] of the published application. New claim 42 is supported by the specification at least at page 7, lines 33-34 and page 9, lines 2-10. New claims 43-45 are supported by the specification at least at page 20, line 33 to page 21, line 7. Reconsideration is respectfully requested.

Information Disclosure Statement

The Examiner provides comments at pages 2-3 of the Office Action of April 18, 2007 (hereinafter, "Office Action") concerning the references cited in the Information Disclosure Statements (IDS) of September 2, 2004, August 31, 2005 and October 15, 2005. The Examiner states that references CA-CD of the August 31, 2005 IDS require re-listing on a Supplemental

IDS Form 1449 which includes the full titles of the references. Attached hereto for the Examiner's signature is a Supplemental Form SB/08 exactly corresponding to the Form SB/08 submitted on August 31, 2005 except that the full titles of references CA-CD have been included.

The Examiner also states that the reference CB of the IDS of September 2, 2004 had no English language translation accompanying the IDS submission and no date was indicated in the corresponding Form SB/08 and therefore this reference was also crossed out by the Examiner. Submitted concurrently herewith is an IDS again listing reference CB of the IDS of September 2, 2004 and accompanied by an English language translation of the relevant portions of the reference CB.

Furthermore, Applicants submit herewith an English language equivalent, EP 1391509 corresponding to reference BB, WO 02/86119 in an Information Disclosure Statement.

Consideration of the entirety of the contents of these references and return of the signed corresponding SB/08 Forms are respectfully requested.

Objections to the Claims

The Examiner objects to claims 31 and 33-38 as being in improper form according to 37 C.F.R. § 1.75(c). (*Id.* at page 3). Claim 31 has been amended herein without prejudice or disclaimer to replace the term "and" with the term "or" thus placing the multiple dependency in alternative form. Claim 34 has also been amended herein without prejudice or disclaimer to remove dependency from claim 33.

Applicants believe these amendments address the Examiner's bases for the objection to these claims and reconsideration and withdrawal of the objection to claims 31 and 33-38 and consideration thereof on the merits are respectfully requested.

The Examiner additionally objects to claims 21-24 for reciting improper grammar. (*Id.*). Although Applicants do not agree that claims 21-24 are in any way improper, to expedite prosecution, claims 21-24 have been amended herein without prejudice or disclaimer to address the grounds of the Examiner's objection, as suggested by the Examiner. Furthermore, the term "piggyBack" has been removed from the claims.

Therefore, reconsideration and withdrawal of the objection to claims 21-24 are also respectfully requested.

Rejections Under 35 U.S.C. § 112, Second Paragraph

Claims 21, 25, 26 and 28 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as their invention. (*See*, *Id.*, at page 4). Claim 25 has been cancelled herein without prejudice or disclaimer, thus obviating the rejection of claim 25. Applicants traverse the rejection as to the remaining claims as set forth herein.

The Examiner states that claim 28 is indefinite because it is uncertain whether the term "is" implies open or closed language. (*Id.*). Although Applicants do not agree that claim 28 is indefinite, to expedite prosecution, claim 28 has been amended without prejudice or disclaimer to recite, "The gene cassette according to claim 27, wherein the 3' terminal portion of the fibroin H chain gene consists of the DNA shown in SEQ ID NO: 24."

The Examiner further states that claim 21 is unclear because it is uncertain how many inverted repeat sequences of the piggyBac transposon are located on either side of the promoter and gene. (*Id.*). The Examiner proposes alternative language for claim 21. (*Id.* at page 5). Although Applicants do not agree that claim 21 is unclear, to expedite prosecution, claim 21 has been amended without prejudice or disclaimer to recite language similar to that proposed by the Examiner.

Reconsideration and withdrawal of the indefiniteness rejection of claims 21, 26 and 28 are respectfully requested.

Rejections Under 35 U.S.C. § 102(b)

Claims 22, 23, 25 and 27 remain rejected under 35 U.S.C. § 102(b) as being anticipated by Zhao et al., *Acta Bioch. Biophys. Sin.*, 33(1):112-116, 2001 (hereinafter, "Zhao et al.") as evidenced by Zhang et al., *Acta Bioch. Biophys. Sin.*, 31(2):119-123, 1999 (hereinafter, "Zhang et al.") and GenBank Accession No. AF226688 (hereinafter referred to as "GenBank"). (*See*, Office Action, at page 5). Claim 25 has been cancelled herein without prejudice or disclaimer, thus obviating the rejection of claim 25. Applicants traverse the rejection as to the remaining claims as set forth herein.

The Examiner states that claims 22, 23 and 27 remain rejected for reasons of record set forth in the Office Action of October 30, 2006. (*Id.*). The Examiner further states that since only claim 21 was amended to recite the limitations of claim 24, the remaining claims remain rejected as previously stated. (*Id.* at page 6).

Although Applicants do not agree that claims 22, 23 and 27 lack novelty, to expedite prosecution, claims 22 and 23 have been amended herein without prejudice or disclaimer to be directed to a gene cassette having an inverted repetitive sequence of a piggBac transposon on either end of the cassette, with various other nucleic acid sequences (parts (2) and (3) of claims 22 and 23) ordered between the two inverted repeats. Furthermore, claims 22 and 23 have been amended herein without prejudice or disclaimer to be limited to a fibroin H chain gene promoter expressed in *Bombyx mori* silk glands (part (2) of claims 22 and 23). This limitation was previously recited in non-anticipated claim 29.

Therefore, claims 22 and 23 are believed to be novel since the cited references fail to disclose all of the limitations of the presently amended claims. "A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." (*See*, *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 U.S.P.Q.2d 1051, 1053 (Fed. Cir. 1987)).

Dependent claim 27 is not anticipated as, *inter alia*, depending from a non-anticipated base claim, claims 22 or 23.

Reconsideration and withdrawal of the anticipation rejection of claims 22, 23 and 27 are respectfully requested.

Rejections Under 35 U.S.C. §§ 102(b) or 103(a)

Claims 26 and 28 stand rejected under 35 U.S.C. § 102(b) as anticipated by, as applied to claims 22, 23, 25 and 27 above, or in the alternative, under 35 U.S.C. § 103(a) as obvious over Zhao et al. as evidenced by Zhang et al. and GenBank, and further in view of Zhou et al., *Nucleic*

Acids Res., 28(12):2413-2419, 2000 (hereinafter, "Zhou"). (*See*, Office Action, at page 6). Applicants traverse the rejection as set forth herein.

The Examiner states that "it is still ambiguous whether the 3′ portion of the fibroin gene can comprise sequence adjacent to SEQ ID NO:24 in the *B. mori* genome." (*Id.*). The Examiner further states that the reasoning behind the present rejection is substantially that set forth in the Office Action of October 30, 2006. (*Id.*).

As already remarked upon, above, with respect to the rejections of claims 22, 23 and 27, Applicants have amended claims 22 and 23 without prejudice or disclaimer to recite limitations, such as the limitation recited in non-anticipated and non-obvious dependent claim 29, which are believed to distinguish the presently claimed invention over the disclosures of the cited references. It is further noted that claims 26 and 28 depend from independent and novel claims 22 and 23, either directly or indirectly.

Therefore, reconsideration and withdrawal of the rejection of claims 26 and 28 are respectfully requested.

Rejections Under 35 U.S.C. § 103(a)

Claims 21-23, 25, 27, 29, 30, 32 and 38 stand rejected under 35 U.S.C. § 103(a) as being unpatentable as obvious over Liu et al., U.S. Patent Application Publication No. 2002/0137211 (hereinafter, "Liu et al.") in view of Zhao et al, Zhang et al. and GenBank. (*See*, Office Action, at page 7). Claim 25 has been cancelled herein without prejudice or disclaimer, thus obviating the rejection as to claim 25. Applicants traverse the rejection as to the remaining claims as hereinafter set forth.

The Examiner cites to the disclosure of Liu et al. to find a similar construct as claimed, except using *B. mori* fibroin L-chain, instead of H-chain. (*Id.*).

Applicants again note that claims 21-23 have been amended herein without prejudice or disclaimer to recite the limitations of claim 29 and to further define the gene cassettes of the present invention. In addition to these newly added distinguishing features, Applicants provide the following comments concerning the lack of motivation to combine the cited references.

Distinguishing Features of the Presently Claimed Invention

in Light of the Disclosures of Zhao et al. & Zhang et al.

The genes disclosed by Zhao et al. and Zhang et al. are similar to the present invention with respect to coupling an exogenous protein gene between genes on the 5' (upstream) side and 3' (downstream) side of silkworm fibroin H chain. However, in contrast to the gene of Zhao et al. and Zhang at al. (which do not have a piggyBac transposon), the gene cassette of the presently claimed invention differs in that it comprises a piggyBac transposon. In the present invention, the possession of a piggyBac transposon results in a significant difference between the presently claimed invention and the technology disclosed in Zhao et al. and Zhang et al. in terms of the effects of the present invention.

Specifically, since the genes disclosed in Zhao et al. and Zhang et al. do not have a piggyBac transposon, the resulting transgenic silkworm is a homologous recombinant in which all of the original fibroin H chains have been replaced with exogenous protein genes. Consequently, the resulting transgenic silkworm is unable to create original fibroin H chain which is essential for forming silk. This being the case, a transgenic silkworm inserted with

only the GFP gene created by Zhang et al. was unable to produce silk. Although Zhao et al. was able to obtain a transgenic silkworm that produced silk by designing a fused protein consisting of a novel fibroin-like gene and GFP, the transgenic silkworm produced by Zhao et al. was only able to produce a fused protein consisting of a fibrous fibroin-like gene and an exogenous protein having a high molecular weight and high crystallinity. This protein of Zheo et al. only dissolved in a solvent having an extremely potent protein denaturing ability, and even when the protein was recovered from the silk gland, Zhao et al. were unable to dissolve the protein in an aqueous solution or buffer. In addition, even after having solubilized the cocoon with a denaturant and the like, it is believed to yield an insoluble precipitate when returned to an aqueous solution or buffer.

On the other hand, the gene cassette of the presently claimed invention differs in that it has a piggyBac transposon. Consequently, since the gene cassette of the presently claimed invention is inserted at a non-specific site of a silkworm chromosome, an inherent homologous recombinant with fibroin H chain does not result. Thus, in a transgenic silkworm obtained in the present invention, the original fibroin H chain and the translation product (protein) of the gene cassette of the present invention are expressed competitively. On the other hand, a transgenic silkworm obtained using the gene disclosed by Zhao at al. and Zhang et al. would be completely different in that the original fibroin H chain is not expressed.

In other words, a transgenic silkworm of the present invention is basically able to produce silk. In addition, since the gene cassette of the present invention has a promoter expressed in the silk gland, it is simultaneously expressed with the original fibroin H chain in the silkworm silk glands, and an exogenous protein designed according to the present invention is produced in cocoon silk in the form of a mixture with fibroin H chain. Consequently, after extracting and solubilizing fibroin H chain protein and exogenous protein from a silk gland, or after having temporarily solubilized silk from the resulting cocoon, only the target soluble exogenous protein can be recovered, thereby making it possible to produce and recover the exogenous protein.

Specifically, the present invention can be used to produce physiologically active protein characterized in that an exogenous protein is recovered after being expressed in a silk gland or silk, whereas it is impossible produce and recover physiologically active protein from a transgenic silkworm produced using the genes disclosed by Zhao et al. and Zhang et al.

Furthermore, in the gene disclosed by Zhao et al. and Zhang et al., the 5' region and 3' region of fibroin H chain merely use an exogenous protein gene as a region for homologous recombination with silkworm fibroin H chain gene, while there is no disclosure regarding expression in the form of a fused protein by matching the frames of the fibroin H chain gene and exogenous protein gene. Since Zhang et al. couple a green fluorescent protein (GFP) gene downstream from an IE promoter originating in cytomegalovirus, and couple a 3' side sequence of fibroin H chain to the 5' side of this gene and the 5' side sequence of fibroin H chain to the 3' side, the GFP and fibroin H chain sequence are facing in the opposite directions, thus preventing

the fibroin H chain and GFP from forming a fused protein. Additionally, the 3' side of the fibroin H chain is not expressed as a protein.

In contrast, the presently claimed invention differs from the disclosures of the cited references in that it is designed so that fibroin H chain gene is partially translated using a promoter expressed in silkworm silk gland, and exogenous protein is expressed in the form of a fused protein with fibroin H chain. As a result, an exogenous protein can be efficiently expressed in silkworm silk gland or cocoon silk by utilizing the expression mechanism of silkworm fibroin H chain gene.

Moreover, the present invention discloses for the first time that the expressed amount of desired protein is improved by coupling an exogenous protein gene to a first exon of a fibroin H chain genomic gene sequence and a second exon linked to a first intron so that their amino acid translation frames match.

In addition, the present invention discloses for the first time that secretion of exogenous protein into a silk gland and the amount of exogenous protein produced in silk is improved by designing a gene structure in which a 3' terminal sequence of fibroin H chain and an exogenous protein gene are coupled so that their amino acid translation frames match.

Specifically, the method disclosed by Zhao et al. and Zhang et al. differs in that only an exogenous protein is expressed, and that exogenous protein is not a fused protein with silkworm fibroin H chain protein. The presently claimed invention provides a gene cassette capable of being expressed as a fused protein with silkworm fibroin H chain protein, and is a method for producing physiologically active protein characterized in that this gene cassette is used to produce an exogenous protein in a silk gland or silk of a transgenic silkworm.

As has been described above, Zhao et al. and Zhang et al. merely disclose a method for producing a transgenic silkworm by homologous recombination using fibroin H chain. It would not be easy for a person of ordinary skill in the art on the basis of these disclosures alone to conceive of a method enabling production of an exogenous protein in a state in which the exogenous protein retains solubility in water and physiological activity in silk. That is, the presently claimed invention could not possibly be derived from the combined disclosures of the cited references, said invention being directed to a method consisting of designing a gene of a fused protein consisting of fibroin H chain and an exogenous protein, producing exogenous protein in a silk gland by controlling expression with a fibroin H chain promoter, producing a fused protein consisting of an exogenous protein and fibroin H chain in the state of being mixed with a fibroin H chain protein originating in a silkworm by using a piggyBac transposon sequence to produce the fused protein in a silkworm, and recovering the exogenous protein from a silk gland or silk.

In addition, Zhao et al. disclose that a cocoon was able to be obtained that is composed of a fibroin-like protein containing fluorescent protein, and Zhang et al. acquired a transgenic silkworm in which a fluorescent protein gene had been inserted into a chromosome. This disclosure reveals that a cocoon was not produced. In contrast, the presently claimed invention enables the production of a cocoon and silk containing physiologically active protein, and that exogenous protein can be recovered from a silk gland or silk, thus provided unexpected results in light of the disclosed technology of Zhao et al. and Zhang et al. These unexpected results could not have been easily conceived by a person of ordinary skill in the art in light of the disclosures

of the cited references since these achievements recited in the presently claimed invention are not disclosed or suggested therein.

Distinguishing Features of the Presently Claimed Invention

in Light of the Disclosure of Zhou et al.

Although Zhou et al. disclose that the entire nucleotide sequence of silkworm fibroin H chain and a fibroin H chain gene are composed of two exons and one intron, Zhou et al. do not disclose or suggest that the expressed amount of an exogenous protein is improved by designing a gene in which an exogenous protein gene is coupled to the second exon so that their frames match, *i.e.* so that the reading frames of both are "in frame." Information useful for expressing an exogenous protein in the form of a promoter sequence and poly A sequence of the fibroin H chain are not disclosed or suggested by Zhou et al. In addition, there is no teaching whatsoever regarding the technology required for acquiring a transgenic silkworm.

More specifically, it would not be easy for a person with ordinary skill in the art to conceive of the presently claimed invention in light of the disclosure of Zhou et al. because the disclosure of Zhou et al. does not disclose or suggest any aspect of a method of enabling production of an exogenous protein in a state in which the exogenous protein retains solubility in water and physiological activity in silk, namely a method consisting of designing a gene of a fused protein consisting of fibroin H chain and an exogenous protein, producing exogenous protein in a silk gland by controlling expression with a fibroin H chain promoter, producing a fused protein consisting of an exogenous protein and fibroin H chain in the state of being mixed with a fibroin H chain protein originating in a silkworm by using a piggyBac transposon

sequence to produce the fused protein in a silkworm, and recovering the exogenous protein from a silk gland or silk, based solely on the gene sequence information of silkworm fibroin H chain disclosed by Zhou et al. In addition, it would also not be easy to conceive the effects of the present invention.

Distinguishing Features of the Presently Claimed Invention in Light of the Disclosure of Liu et al.

The information disclosed in Lui et al. is similar to the presently claimed invention in that both methods utilize a piggyBac transposon to produce a transgenic silkworm. However, in contrast to the gene described by Lui et al., disclosing the coupling of silkworm fibroin L chain and the spider dragline silk protein gene, the gene of the presently claimed invention differs in that it couples silkworm fibroin H chain and an exogenous protein gene. The gene described by Lui et al. uses a silkworm fibroin L chain promoter as a promoter expressed in silkworm silk glands, and uses a fibroin L chain terminator for the terminator. Namely, Lui et al. do not make any disclosure whatsoever regarding a method for producing exogenous protein using silkworm fibroin H chain.

The gene of the present invention uses silkworm fibroin H chain promoter as the promoter expressed in silkworm silk glands, and uses a fibroin H chain terminator for the terminator. On the other hand, the present invention disclosed for the first time that the expressed amount of an exogenous protein is improved by coupling an exogenous protein to a first exon of a fibroin H chain genomic gene sequence and a second exon linked to a first intron so that their amino acid translation frames match. In addition, the present invention also

disclosed for the first time that secretion of exogenous protein into a silk gland and the amount of exogenous protein produced in silk is improved by designing a gene structure in which a 3'-terminal sequence of fibroin H chain and an exogenous protein gene are coupled so that their amino acid translation frames match. Moreover, the present invention also discloses for the first time that the expressed amount of an exogenous protein is improved considerably by coupling to the 5' upstream region (approx. 5 kb) of fibroin H chain promoter.

As a result of this frame correction, the promoter expressed in silkworm silk glands in claims 21 to 23 is limited to fibroin H chain promoter, claim 29, describing L chain promoter as a promoter capable of being expressed in silkworm silk glands has been deleted, and the L chain and serine poly A chain have been deleted with respect to the poly A sequence of claim 30. In addition, a sentence indicating that the exogenous protein is recovered in an aqueous solution after being expressed has been added to claim 34. Thus, differences between the technology described by Lui et al. and the present invention are clearly distinguishable.

One distinguishing feature of the presently claimed invention over that disclosed in Liu et al. is that although Lui et al. report that a fused protein of a full length fibroin L chain and a spider dragline silk protein was confirmed in silk gland and silk, this is not disclosed as being recovered in an aqueous solution. Fibroin L chain, according to Liu et al., is secreted into silk glands in a state of being covalently bonded on a 1:1 basis with fibroin H chain, and since it is spun in the form of silk, the fused protein described by Lui at al. ought to be coupled with the original silkworm fibroin H chain. Since fibroin H chain is a protein that is insoluble in water, and since the fused protein described by Lui et al. does not dissolve in an aqueous solution, it is not believed to be recoverable in an aqueous solution.

In addition, Lui at al. disclose that a fused protein of fibroin L chain and a spider dragline silk protein constitute 30% of the total silk protein. Since the technology of Lui at al. uses a transposon, the original silkworm fibroin H chain and L chain are expressed competitively. As disclosed by Zhou et al. (*Nucleic Acids Res.*, 28(12), 2413-2419 (2000)), a fused protein consisting of fibroin H chain, fibroin L chain and a spider dragline silk protein composes silk by being expressed in silk glands in the state of being bonded by sulfide bonds in a 1:1 ratio. When considering these facts in light of the fact that the molecular weight of fibroin H chain is about 350,000 Daltons and the molecular weight of the fused protein of Lui at al. is about 85,000 Daltons, even if a fused protein of fibroin L chain and a spider dragline silk protein were maximally expressed, a value of 19.5% or more as percent by weight would be theoretically impossible. The content of fused protein disclosed by Lui at al. has been indicated to be able to be interpreted as 30 mol % of total silk protein.

On the other hand, the presently claimed invention discloses that the amount of exogenous protein produced is measured after recovering the exogenous protein from a silk gland in an aqueous solution by homogenizing in 100 mM phosphate buffer (Example 18). In addition, it also discloses that silk from a silk gland is dissolved in 60% LiSCN aqueous solution followed by diluting with phosphate-buffered PBS and measuring the amount of exogenous protein in the solution. Namely, the fused protein consisting of fibroin H chain and exogenous protein obtained in the present invention is soluble in water, and therefore can be recovered in an aqueous solution. This being the case, the present invention makes it possible for the first time to produce a physiologically active protein in this manner. In addition, the present invention discloses that fibroin H chain and exogenous protein recovered in an aqueous solution

constituted 1.0 to 4.9% of the cocoon weight (Example 18). When considering the molecular weights of simultaneously produced fibroin H chain and fibroin L chain, this means that exogenous protein is produced at 15 to 40 mo1% of the total silk protein.

Particularly, even if Lui et al. disclose a gene which is silkworm fibroin L chain coupled with spider dragline silk protein, along with the acquisition of a transgenic silkworm by utilizing this gene, the present invention differs therefrom in that a gene of a fused protein consisting of fibroin H chain and an exogenous protein is designed, exogenous protein is produced in a silk gland by controlling expression with a fibroin H chain promoter, and a fused protein consisting of an exogenous protein and fibroin H chain is produced in a silkworm in the state of being mixed with a fibroin H chain protein originating in a silkworm by using a piggyBac transposon sequence. Moreover, the method for producing physiologically active protein has greater effects in that a technology is provided that enables recovery of an exogenous protein from a silk gland or silk in an aqueous solution.

Thus, for at least these reasons, claims 21-23, 27, 29, 30, 32 and 38 are believed to be non-obvious in light of the cited references.

Reconsideration and withdrawal of the obviousness rejection of claims 21-23, 27, 29, 30, 32 and 38 are respectfully requested.

CONCLUSION

If the Examiner has any questions or comments, please contact Thomas J. Siepmann, Ph.D., Registration No 57,374, at the offices of Birch, Stewart, Kolasch & Birch, LLP.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to our Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under § 1.17; particularly, extension of time fees.

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Respectfully submitted,

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Attachments: Supplemental Information Disclosure Statement (IDS) Form SB/08
corresponding to the IDS submitted on August 31, 2005 and
providing full titles to references CA-CD and IDS submitted on
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with an original Japanese reference.